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RESEARCH ARTICLE

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Validation of glutathione quantitation from STEAM spectra against edited ^{1}H NMR spectroscopy at 4T: application to schizophrenia

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Abstract *Objective*: Quantitation of glutathione (GSH) in the human brain in vivo using short echo time ^{1}H NMR spectroscopy is challenging because GSH resonances are not easily resolved. The main objective of this study was to validate such quantitation in a clinically relevant population using the resolved GSH resonances provided by edited spectroscopy. A secondary objective was to compare several of the neurochemical concentrations quantified along with GSH using LCModel analysis of short echo time spectra in schizophrenia versus control. *Materials and Methods*: GSH was quantified at 4T from short echo STEAM spectra and MEGA-PRESS edited spectra from identical volumes of interest (anterior cingulate) in ten volunteers. Neurochemical profiles were quantified in nine controls and 13 medicated schizophrenic patients. *Results*: GSH concentrations as quantified using STEAM, $1.6 \pm 0.4\,\mu$mol/g (mean $\pm$ SD, $n = 10$), were within error of those quantified using edited spectra, $1.4 \pm 0.4\,\mu$mol/g, and were not different ($p = 0.4$). None of the neurochemical measurements reached sufficient statistical power to detect differences smaller than 10% in schizophrenia versus control. As such, no differences were observed. *Conclusions*: Human brain GSH concentrations can be quantified in a clinical setting using short-echo time STEAM spectra at 4T.

Keywords Glutathione · Magnetic resonance spectroscopy · Schizophrenia

Introduction

Improvements in proton nuclear magnetic resonance (^{1}H NMR) sensitivity and resolution attained at higher magnetic field strength [1,2] have lead to expansion of the list of neurochemicals that can be quantified in vivo beyond the typically reported N-acetyl aspartate (NAA), creatine (Cr), choline (Cho), and sometimes inositol (Ins) and the sum of glutamate and glutamine (Glx). One additional neurochemical that can be detected is glutathione (GSH). Although the small, coupled resonances from glutathione (reduced, GSH) are not resolved from overlapping neurochemical resonances at 4T, they are detected using deconvolution software such as Linear Combination Model (LCModel, [3]) to uncover them. Whereas visual affirmation of accurate quantitation as such is absent, GSH resonances can be resolved and unambiguously assigned in the human brain using edited ^{1}H NMR spectroscopy [4,5]. Fully resolved GSH resonances were recently measured using MEGA-PRESS (MEscher – GArwood-Point RESolved Spectroscopy) editing, which was highly specific for unequivocal detection of the cysteine β-CH_2 resonance of GSH at 2.95 ppm [5]. Sensitivity of GSH detection as such was sufficient for measuring the approximated 1.3 μmol/g concentration, which was in excellent agreement with other MRS measurements (2–5 mmol/L) [4] as well

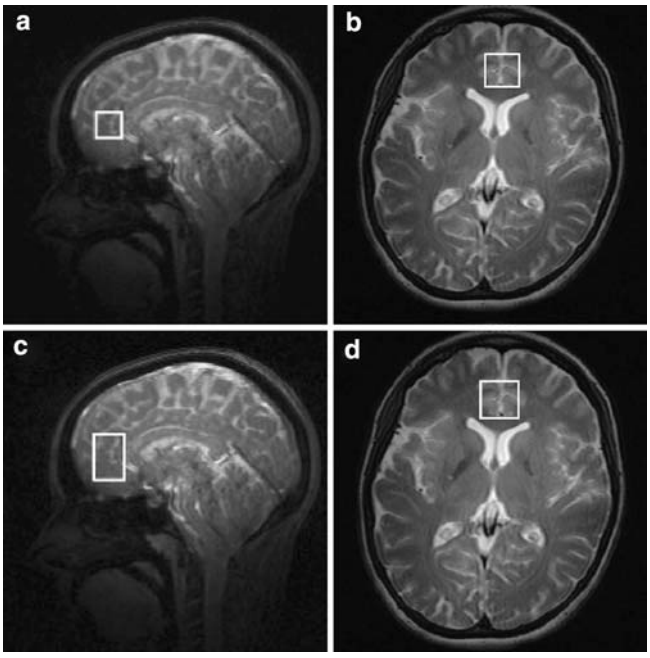

Fig. 1 **a** Scout sagittal image (GEMS, TR = 50 ms, TE = 4 ms, 256×128 matrix, no averaging, 5 mm slice thickness) and **b** localizer transverse image (RARE, TR = 4.0 s, TE = 60 ms, echo train length = 8, 256×128 matrix, two averages, 2 mm slice thickness, one slice of seven) illustrating the $2\times2\times2\,cm^3$ VOI in the anterior cingulate. **c** and **d** Duplicate images for illustration of the $2.3\times2.2\times3.3\,cm^3$ VOI containing the anterior cingulate

as the lower end of GSH concentrations reported in human brain via biochemical methods, i.e., 1 [6], 1–3 [7], and 1–10 [8] mM. The main goal of this study was to validate reliable GSH quantification from short-echo time stimulated echo acquisition mode (STEAM) spectra against MEGA-PRESS edited spectra in a clinically relevant setting.

A recent study reported decreased GSH in cerebrospinal fluid in patients with schizophrenia as well as decreased brain (52%, $p = 0.001$, frontal cortex) GSH levels as measured with in vivo edited magnetic resonance spectroscopy [9]. GSH and ascorbate are the most concentrated non-enzymatic antioxidants in the central nervous system [10]. Studies suggest that oxidative stress [11] and altered activity of GSH peroxidase, an important antioxidant enzyme [12] may be involved in the pathogenesis of schizophrenia [9]. Additional metabolites of interest in the study of schizophrenia, such as glutamate and glutamine can be quantified concurrently with GSH from short-echo time STEAM spectra [13]. Since the various GSH concentrations present in schizophrenia versus control served as a good data set for the validation study, and quantifying the neurochemical profile in schizophrenia would contribute to understanding the disease, a secondary goal of this study was to measure neurochemical concentrations in medicated schizophrenia versus control.

Methods

Protocol

Test–retest repeatability of the neurochemical profile as quantified from short echo time STEAM spectra was assessed by measuring the profile from an $8\,cm^3$ volume of interest (VOI) in the anterior cingulate (Fig. 1a, b) in three volunteers, three consecutive times, on three different days. One neurochemical profile was disregarded due to atypically high Cramer–Rao lower bounds (CRLB > 15% for GSH and Gln quantitation), and another set of three profiles was not completed due to scheduling conflicts. Among the 20 in the basis set, neurochemicals for which the average CRLB was 12% or less in the test-retest study were considered reliable for further investigation. For the next phase of the study, patients and new controls were presented for spectroscopy in a blinded fashion. From these subjects: a STEAM spectrum was acquired from an $8\,cm^3$ VOI in the anterior cingulate cortex (Fig. 1a, b) for comparison of schizophrenia versus control, an edited spectrum was acquired from a $17\,cm^3$ VOI containing the anterior cingulate (Fig. 1c, d) for validation against another STEAM spectrum, which was acquired from the same $17\,cm^3$ VOI. Ten subjects (three patients, seven controls) tolerated the 90 min scan time required for measurement of all three spectra. Twelve additional subjects (total of 13 patients, nine controls) tolerated at least 30 min, allowing measurement of one STEAM spectrum ($8cm^3$). The $8\,cm^3$ VOI was selected in order to localize the neurochemical profile to the anterior cingulate. Based on previous studies [9], the larger volume of interest was expected to provide sufficient signal to noise for edited spectroscopy. In order to match experimental conditions for validation of GSH quantitation, an additional STEAM spectrum was acquired from the larger volume of interest. The inclusion criteria for each neurochemical concentration measured from STEAM spectra was a CRLB of 20% or less. The study was unblinded after data were processed. For comparing GSH concentrations measured using the two techniques ($17\,cm^3$ VOI), statistical significance was calculated using a two tailed student's t test for samples of equal variance. For comparison of neurochemical concentrations in schizophrenia versus control (two treatment parallel design), the smallest difference in means that could have been detected with an 80% probability (two sided 5% significance level) was calculated based on the sample size (total) and standard deviation (average) in the patient versus control study for each neurochemical.

Human subjects

Three normal volunteers (one male, age 20 ± 3, mean ± SD) gave informed consent for the test–retest portion of the study. All experiments were conducted according to the procedure approved by the institutional review board. For the blinded portion of the study, patients ($n = 13$, eight male, age 26 ± 5, schizophrenia, 11; schizoaffective disorder, 2) were diagnosed with the Structured Clinical Interview for DSM-IV Axis I Disorders –

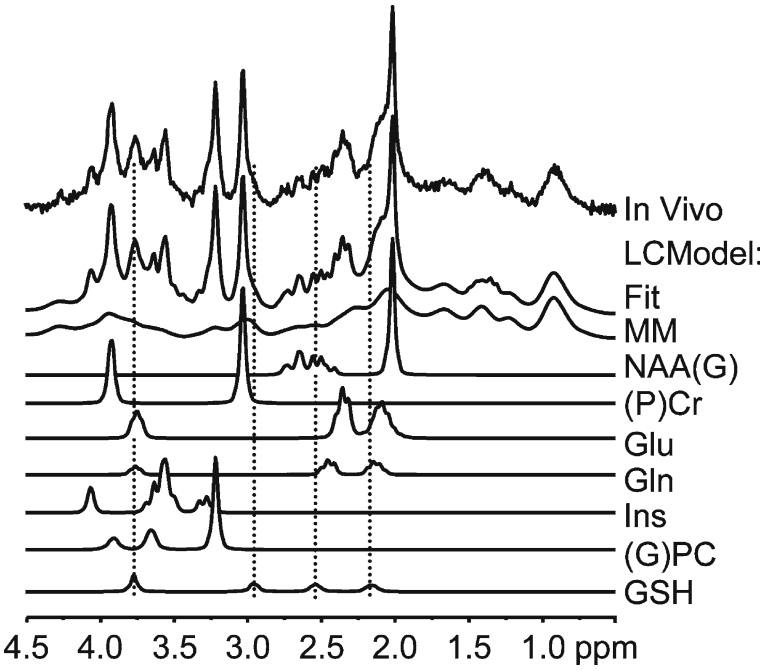

Fig. 2 In vivo STEAM spectrum (VOI = 8 cm^3, TE = 5 ms, TR = 4.5 s, TM = 42 ms, NEX = 256) and LCModel fit, illustrating metabolite contributions to neurochemical profile: *MM* macromolecules, *Gln* glutamine, *Ins* inositol, *Glu* glutamate, *(P)Cr* (phospho)creatine, *NAA(G)* N-acetyl aspartate(glutamate), *GSH* glutathione, and *(G)PC* (glycerol)phosphorylcholine

Patient Edition (SCID-P [14]). All patients were medicated. Controls ($n = 9$, four male, age 25 ± 5) were screened for Axis I disorders with the SCID – Nonpatient Edition (SCID-NP [14]).

Spectroscopy and quantitation

Imaging and spectroscopy were performed on a 4 T, 90 cm bore magnet (Oxford Magnet Technology, Oxford, UK) interfaced to a Varian INOVA spectrometer (Varian, Palo Alto, CA) equipped with gradients capable of switching to 40 mT/m in 400 μs (Sonata, Siemens, Erlangen, Germany) and a transverse electromagnetic (TEM) volume coil. Subjects were positioned supine. The protocol for each volunteer began with a scout sagittal image (GEMS, TR = 50 ms, TE = 4 ms, 256×128 matrix, no averaging, 5 mm slice thickness) followed by localizer multislice transverse images (RARE, TR = 4.0 s, TE = 60 ms, echo train length = 8, 256×128 matrix, two averages, 2 mm slice thickness, seven slices separated by 0.5 cm) for selection of a volume of interest in the most anterior portion of the cingulate cortex (Fig. 1). Shimming of all first- and second-order coils was achieved using FAST(EST)MAP [15], resulting in water line widths of 6–11 (average 8) Hz in the 8 cm^3 VOI. The same shim settings were used for the 17 cm^3 VOI, wherein the average linewidth was 1 Hz larger. Water signal was suppressed by variable power radiofrequency (RF) pulses with optimized relaxation delays (VAPOR) [16]. When motion was suspected, imaging and shimming were repeated as necessary. An image was acquired at the end of each study to affirm VOI placement. STEAM spectroscopy [17] (TE = 5 ms, TR = 4.5 s, TM = 42 ms, NEX = 256) and quantitation of metabolite concentrations, or the neurochemical profile [18], were performed in both the 8 cm^3 VOI and the 17 cm^3 VOI. MEGA-PRESS difference editing and quantitation of the GSH edited spectra were performed as previously described [5].

Results

To illustrate the challenge encountered in separating GSH resonances from overlapping resonances, Fig. 2 shows a STEAM spectrum from an 8 cm^3 VOI in a normal volunteer as well as neurochemical fits. Note that unlike other neurochemicals, GSH does not contribute a unique resonance, despite statistical affirmation of successful separation, i.e. CRLB less than 15% [3]. The LCModel fit is nearly identical to the in vivo spectrum, indicating that LCModel was successful in modeling the in vivo spectrum as a linear combination of basis spectra.

In contrast to the overlapping resonances appearing in the STEAM spectrum (Fig. 2), Fig. 3 illustrates fully resolved GSH resonances via a representative MEGA-PRESS difference edited spectrum. Co-edited NAA (≤2.8 ppm) was well separated from GSH (≥2.8 ppm). Quantification of GSH using LCModel analysis of this in vivo spectrum was successful as indicated by: the low CRLB (14%), the close match between the in vivo spectrum and the fit, and the noise predominated fit residual.

Agreement ($p > 0.3$) between GSH concentrations quantified by STEAM, 1.6 ± 0.4 μmol/g (mean ± SD, $n = 10$) versus edited spectroscopy, 1.4 ± 0.4 μmol/g (mean ± SD, $n = 10$) in matched volumes of interest is detailed in Table 1 and illustrated in Fig. 4. Given the variance of the

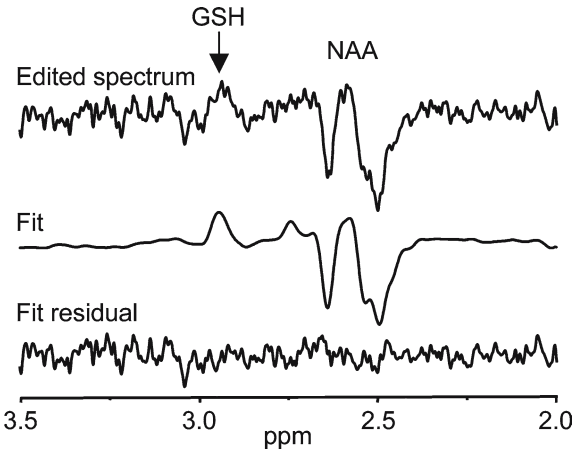

Fig. 3 In vivo MEGA-PRESS spectrum edited for GSH (NEX = 512, TR = 4.5 s, TE = 68 ms, VOI = 17 cm^3) and results of LCModel analysis (fit and residual). NAA co-edits with GSH as expected but is fully resolved

Table 1 GSH Concentrations

Method	Mean	SD
STEAM	1.6	0.4
MEGA-PRESS	1.4	0.4

GSH concentrations (μmol/g) quantified using STEAM and MEGA-PRESS edited spectra (17 cm^3 VOI) for validation, $n = 10$, $p = 0.4$

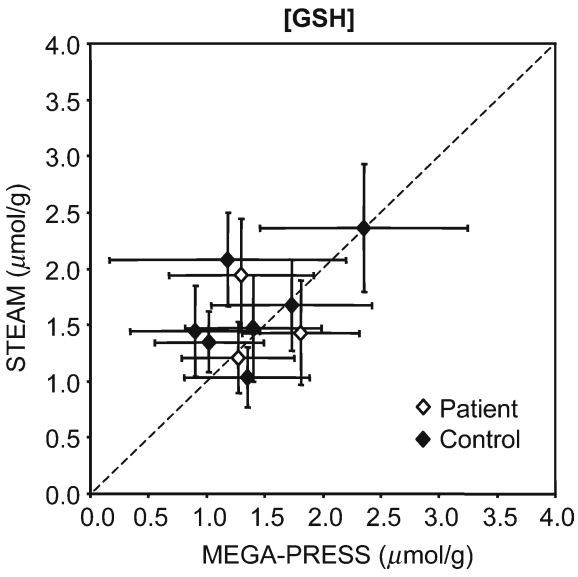

Fig. 4 GSH concentrations measured using short echo time STEAM versus MEGA-PRESS edited ^{1}H MRS in identical volumes of interest, VOI = 17cm^3. *Closed symbols* represent controls whereas *open symbols* represent patients. Error *bars* represent ± 2 * CRLB, or the 95% confidence interval for measuring difference in measured values using LCModel. The unity line representing agreement is drawn for reference

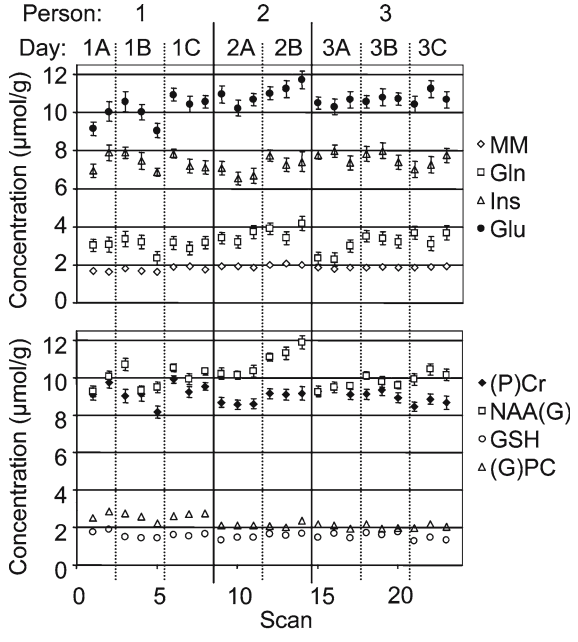

Fig. 5 Test–retest repeatability results, VOI = 8 cm^3. Neurochemical concentrations determined in three people (1–3) on 3 days (A–C), repeated three times each day. Error *bars* represent ±CRLB and are not shown where they fall within the dimension of the symbol. Summary statistics are provided in Table 2

data relative to measurement error, the range of GSH concentrations encountered was not sufficient for establishing correlation between the two measurement techniques (Pearson $r = 0.5$, Spearman's rho = 0.3). Nevertheless, error bars indicating twice the Cramer–Rao lower bound, the 95% confidence interval for separating concentrations measured using LCModel [19] intersected the unity line for 9 of 10 subjects. For the tenth subject, the error bar missed by only 0.02 μmol/g, which becomes 0.0 μmol/g when reported with the appropriate number of significant figures. No significant difference was detected (paired t-test, $p = 0.2$, $t = -1.3$) between GSH concentration measured using STEAM versus MEGA-PRESS editing in the ten individuals.

The results of the study designed to measure the test-retest repeatability of the entire neurochemical profile (8 cm^3 VOI) are illustrated in Table 2 and Fig. 5. These data illustrate the variation in neurochemical concentrations measured in the control group, providing a basis for estimating the smallest difference that can be detected in patients versus controls. Repeatability and CRLB agreed with those reported in a similar study [20].

Finally, the results of comparing the neurochemical profile quantified in Schizophrenia versus control are summarized in Table 3, along with the smallest difference that would have been observed with an 80% probability. None of the following neurochemical concentrations: N-acetyl aspartate(glutamate), (phospho)creatine, glutamate, glu-

Table 2 Test retest

Metabolite	Mean	SD	Average CRLB (%)
NAA(G)	10.1	0.7	3
(P)Cr	9.1	0.4	3
Glu	10.5	0.6	4
Gln	3.2	0.5	11
GSH	1.6	0.2	12
Ins	7.4	0.4	5
(G)PC	2.3	0.3	5
MM	1.9	0.1	3

Neurochemical concentrations (μmol/g) measured in test-retest portion of study (8 cm^3 VOI), $n = 23$

tamine, GSH, inositol, (glycerol)phosphorylcholine and macromolecules were measured with enough statistical power ($p \geq 0.2$) to detect differences smaller that 10%. As such, no differences were observed in schizophrenia versus control. No significant differences or trends ($p > 0.05$) for gender were observed.

Discussion

In order to test our ability to separate GSH resonances from overlapping resonances in short echo time STEAM spectra and therefore quantify GSH concentration correctly, we compared STEAM measured GSH concentrations with those measured using edited spectroscopy, where spectral overlap is not a concern. Given that in our former studies, GSH quantification via edited spectroscopy produced concentrations in good agreement with the preexisting literature [5], and that the concentrations detected using STEAM versus editing agreed within measurement error, our data show that GSH concentrations were neither over- nor under-quantified using STEAM spectroscopy.

Glutathione quantitation via STEAM spectra utilized all four undistorted GSH resonances, each appearing at the correct offset and in correct proportion. Components of spectral quality that contributed to accurate GSH quantitation in this study include: high signal to noise, high chemical shift resolution, minimal water contribution, absence of out-of phase contributions, and flat baseline.

Whereas differing transverse relaxation rates (T_2) have negligible influence at the short echo time used for STEAM spectroscopy, quantification of GSH at the long echo time used for edited spectroscopy depends upon relative relaxation rates. T_2 were expected to have a negligible effect on quantitation relative to the precision achieved in this study and the large concentration changes reported in schizophrenia [9]. For example, if the T_2 of NAA were 120 ms, a 30% error (the SD of the edited GSH concentrations) in GSH via MEGA-PRESS quantitation would result from a GSH T_2 in the large range of 74–225 ms. A long repetition time (TR = 4.5 s) was used in order to minimize the influence of longitudinal relaxation (T_1) on quantitation. GSH levels are reportedly more than 50 times higher than levels of the oxidized form [21, 22], GSSG in normal white matter where GSH is predominant [10]. Therefore, resonance intensity was assigned to reduced GSH.

While this study did not achieve sufficient power to detect neurochemical differences smaller than 10% in schizophrenia versus control, it did achieve sufficient power for comparison with other studies. The previous finding of a 52% smaller GSH concentration in the medial prefrontal cortex of patients with schizophrenia [9] was not measured in our study of the anterior cingulate. Although the previously measured changes in the medial prefrontal cortex may have been region specific and not reflected in the anterior cingulate, the constancy of GSH measured in our study in which all patients were medicated may suggest

Table 3 Schizophrenia versus control

Metabolite	Cntrl. mean	Cntrl. SD	Pt. mean	Pt. SD	p	Min. Δ
NAA(G)	10.1	0.8	10.1	1.0	1.0	1.3
(P)Cr	8.5	0.7	8.7	0.7	0.7	0.9
Glu	10.0	0.7	10.2	0.9	0.5	1.1
Gln	3.1	0.7	3.1	0.8	1.0	1.1
GSH	1.6	0.2	1.5	0.3	0.4	0.4
Ins	7.4	0.8	7.9	0.8	0.2	1.0
(G)PC	2.3	0.1	2.3	0.3	0.6	0.4
MM	1.9	0.1	1.9	0.2	0.7	0.3

Neurochemical concentrations and standard deviations (μmol/g) measured in 13 patients (Pt.) and nine controls (Cntrl.) in 8 cm^3 VOI. For patients, $n = 13$ except for Gln ($n = 12$) and GSH ($n = 11$), where points with CRLB > 20% were removed. Similarly, $n = 9$ for controls except for Gln ($n = 8$). Summary statistics include the minimum change in means that would have been detected with a probability of 80% (Min. Δ)

a normalizing effect of medication, since the patients in the study by Do et al. [9] varied from medicated to drug naïve. Brain GSH content has also been shown to change in human PD [23] (37–89% decreases) and developing rat cortex [10] (60% increase). Several studies of schizophrenia have reported the following decreases in NAA content, most of which would have been sizable enough for detection in our study: 31% ($p = 0.005$) in prefrontal cortex [9], 12% ($p = 0.005$) in frontal lobe [24], 17% ($p < 0.05$) [25] and 56% ($p < 0.001$) [26] in dorsolateral prefrontal cortex (DPFC). Of these, the one that showed the greatest decrease in NAA [26] was the only one that corrected for varying gray matter content, which has been shown to improve ability to detect changes [27]. Since cortical atrophy has been measured in schizophrenia [12], correction for neuron and water content could increase our ability to measure changes. Techniques that have been proposed for such correction include brain tissue segmentation, external referencing, and assessment of water compartmentalization via measurement of water signal at multiple echo times [28]. Our study did not achieve enough power to detect the previously reported 15% increase in glutamine ($p = 0.03$) [13] in schizophrenia versus control. Although our study did not affirm the findings of a recent study [26] in which large decreases in Cr (60%, $p < 0.001$) and Cho (54%, $p < 0.001$) in the DPFC were reported, it did agree with prior studies in which no changes in Cr (left anterior cingulate, [13]) or Cho (left anterior cingulate [13], frontal lobe [24], DPFC [25]) were observed. Similar to GSH in the medial prefrontal cortex versus the anterior cingulate, the changes measured in the DPFC may not have been reflected in the anterior cingulate.

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